Validation of Dual X-Ray Absorptiometry for Body-Composition Assessment of Rats Exposed to Dietary Stressors

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Evidence of the validity and accuracy of dual x-ray absorptiometry (DXA) to measure soft-tissue composition of laboratory rats with altered body composition associated with nutritional perturbations is lacking. We compared DXA determinations made in prone and supine positions with measurements of chemical composition of 49 male, weanling Sprague-Dawley rats that were fed the basal AIN-93 growth diet, were fed the basal diet modified to contain 30% fat, were fasted for 2 d, were limit fed 6 g of the basal diet daily for 1 wk, or were treated with furosemide (10 mg/kg intraperitoneally 2 h before DXA). DXA produced similar estimates of body mass and soft-tissue composition in the prone and supine positions. DXA estimates of body composition were significantly correlated with reference composition values (R^2 = 0.371–0.999). DXA discriminated treatment effects on body mass, fat-free and bone-free mass, fat mass, and body fatness; it significantly underestimated body mass (1% to 2%) and fat-free and bone-free mass (3%) and significantly overestimated fat mass and body fatness (3% to 25%). The greatest errors occurred in treatment groups in which body mass was diminished and body hydration was decreased. These findings suggest that DXA can determine small changes in fat-free, bone-free mass in response to obesity and weight loss. Errors in DXA determination of fat mass and body fatness associated with extra corporeal fluid and dehydration indicate the need for revision of calculation algorithms for soft-tissue determination. *Nutrition* 2001;17:607–613. ©Elsevier Science Inc. 2001

KEY WORDS: obesity, weight loss, dehydration, in vivo method

INTRODUCTION

The development and application of dual x-ray absorptiometry (DXA) for assessment of body composition in clinical and nutrition studies have been limited principally to humans.[1] This technique, however, offers similar opportunities in nutrition and metabolic studies of laboratory rodents, in particular rats. The effort and cost generally limit routine body-composition assessment in nutrition studies using rats. Many of the techniques commonly used to measure rat body composition have practical limitations. Densitometry,[2] total body water (TBW) by isotope dilution,[3] single-frequency bioelectrical impedance,[4] and total body conductivity[5] rely on the two-component model (e.g., fat and fat free), which depends on assumptions regarding the constant chemical composition of the fat-free body.[6] These methods are indirect and can produce unreliable estimates of body composition, particularly when nutritional interventions alter the chemical composition of the fat-free body. An alternate approach is the use of direct chemical analysis of the body with chemical extractions. This method provides accurate measurements of water, protein, fat, and ash contents of the rat and is considered the ultimate reference method for body-composition determination.[7] Although this chemical approach is precise and accurate, it is time-consuming and tedious and requires killing the rat, which precludes its use in longitudinal studies of dietary treatments and interventions.

As a non-destructive method, DXA may be useful for body-composition assessment of laboratory rats. Originally developed to measure bone-mineral content and density of humans, DXA has been used successfully in studies of the whole body and regional bone in rats.[8,9] Evaluation of DXA to measure soft-tissue composition of laboratory rats has been limited. As compared with chemical analysis, DXA significantly overestimated body fat in normal-weight[10] and genetically obese[11] rats. Whether DXA can discriminate small changes in the soft-tissue composition (e.g., fat and fat free, bone free) of rats, similar to that encountered in humans with mild obesity, mild and moderate dehydration as seen in caloric restriction and weight loss, remains to be determined.

The purpose of this study was to determine the precision and accuracy of DXA, relative to chemical analyses, and measure the alterations in the various components of soft-tissue composition of rats exposed to dietary manipulations designed to alter body fatness and hydration. A second goal was to ascertain the effect of body position during DXA on the estimates of body composition.

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MATERIALS AND METHODS

Animal Care and Use

The protocols for this study were approved by the Grand Forks Human Nutrition Research Center (GFHNRC) Animal Care Committee. Forty-nine male Sprague-Dawley weanling rats (40 to 45 g) were purchased from Harlan (Madison, WI). All rats were fed the same nutritionally adequate diet[12] until they attained body weights of approximately 300 g before assignment to treatment groups. The rats were housed in the GFHNRC animal-care facility and kept in individual, wire-bottom, stainless-steel hanging cages. A 12-h light, 12-h dark cycle was used throughout the study. Accepted animal-care practices as outlined by the National Research Council[13] and the US Department of Agriculture were followed for maintaining proper environmental and health conditions. Feeding, sanitation, and animal-health checks were performed daily by trained animal-care technicians. Rat body weights were obtained twice weekly. The rats were maintained in the animal-care facility until they were transported to the DXA laboratory for scanning. All rats were killed after completion of DXA.

Diets and Treatments

The rats were randomly assigned to one of five treatment groups: control, fat fed (FAT), fasted for 2 d (FST), limit fed (LMF), and treated with furosemide (DH). The animals were fed a powdered, purified growth diet[12] containing all essential minerals and vitamins and provided deionized, triple-distilled water. The FAT group received a modified diet containing 30% fat. Except for the LMF group, animals were fed ad libitum. The LMF rats were fed 6.0 g of the powdered AIN-93 diet daily for 1 wk before the DXA measurements. This food intake represented an approximately 50% to 70% reduction in daily caloric intake compared with food intakes measured in the control group. No diet was given to the FST group for 2 d before scheduled DXA scans; water was available ad libitum. The DH group was injected intraperitoneally with furosemide (10 mg/kg; Hoechst, Marion, Roussel, Kansas City, KS, USA). The furosemide was administered 2 h before the scheduled DXA scan. Pre- and postadministration body weights were recorded to determine the amount of urine loss. Food and water were restricted after furosemide administration.

DXA Analysis

The DXA scans were made with a Hologic QDR 2000W (Bedford, MA, USA) instrument. The manufacturer's small-animal module software (version 5.7c) was used to determine fat mass (FM), bone-mineral content, and fat-free, bone-free mass (FFBFM) from the raw scan data. A single-beam collimator and platform attenuator constructed from acrylic resin (Plexiglas) were used with the system. The attenuator platform was necessary to modulate the intensity of the x-ray exposure of the rats and ensure that the x rays reaching the detector were in the operating range of the system. Correction for the density of the attenuator platform was made during the final analysis of the DXA scan data. Calibration of the DXA system was conducted by using a step acrylic (Plexiglas) and aluminum phantom that represents a broad range of soft-tissue and bone densities, respectively. The rat platform was the same height as the lowest step on the calibration bar so that the rat body was in the range of the calibration of the DXA instrument. The DXA system was calibrated at the start of each scan session for an individual rat with the calibration bar and attenuation platform (Fig. 1).

Each rat was immobilized for DXA by anesthesia with a mixture of ketamine (50 mg/kg; Ketaset, Aveco, Fort Dodge, IA, USA) and xylazine (10 mg/kg; Rompun, Mobay, Shawnee, KS, USA) administered intraperitoneally. The anesthetized state persisted at least 140 min and allowed sufficient time to accomplish multiple DXA scans, as described below. Body weight of the anesthetized rat was measured to the nearest 0.1 g. The rat was carefully positioned on the attenuator platform in the prone or supine position with attention to body alignment. The fore- and hindlimbs of the rat were placed perpendicular to the long axis of the body during a prone scan. A slight inferior dorsal abduction of the limbs was used for the supine position. The tail was positioned in a curve toward the head so that it would lie approximately 10 cm from the body and be contained within the scan area. A small piece of transparent adhesive tape was used at the tip of the tail to maintain it in this position (Fig. 2).

Triplicate prone and supine scans were completed for each rat.

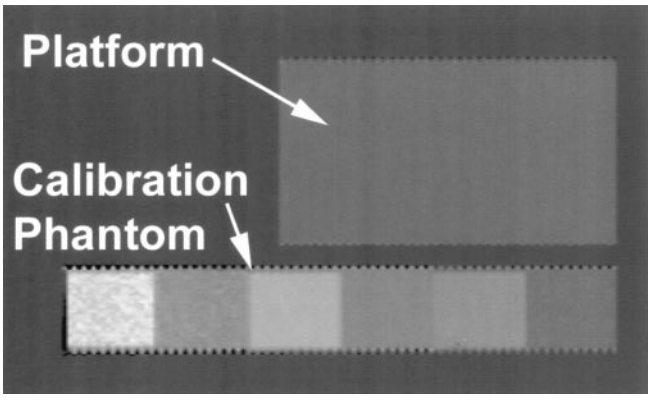

FIG. 1. Calibration bar and attenuation platform used in dual x-ray absorptiometry of rats.

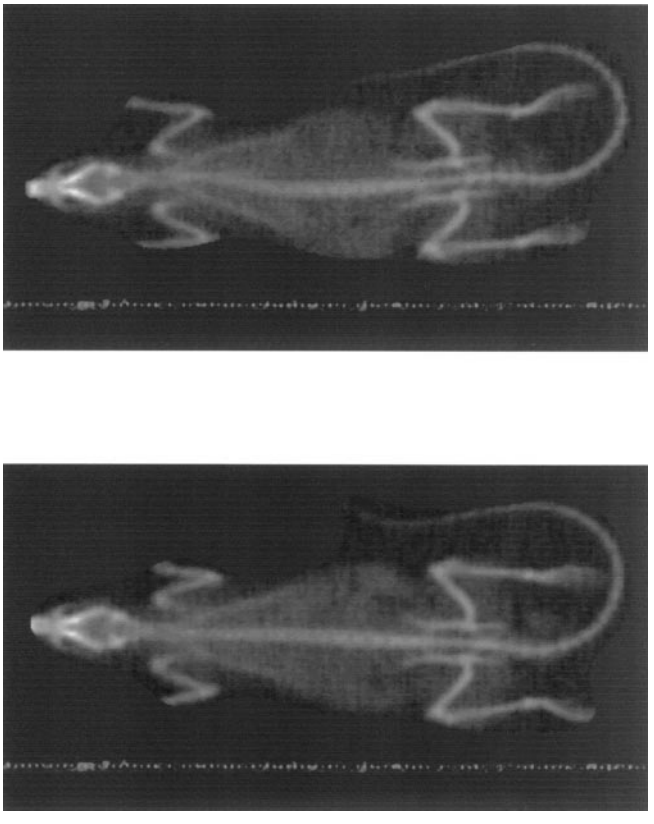

FIG. 2. Prone (top) and supine (bottom) scans of a rat.

TABLE I.

VARIABILITY* OF BODY-COMPOSITION ESTIMATES WITH DUAL X-RAY ABSORPTIOMETRY BY POSITION AND TREATMENT

	Treatment									
	Control		FAT		FST		LMF		DH	
Variable	P	S	P	S	P	S	P	S	P	S
Mass	0.67	0.55	0.49	0.45	0.23	0.25	0.21	0.19	0.17	0.25
FFBFM	0.83	1.25	1.14	0.67	0.77	0.93	1.13	0.84	0.51	0.71
FM	3.65	5.31	4.11	2.63	3.99	5.45	8.89	7.10	2.78	3.13
%FAT	5.21	5.78	4.62	4.89	4.32	4.98	6.78	6.94	3.54	3.85

* Coefficient of variation (%).

DH, treated with furosemide; FAT, high-fat diet; %FAT, percentage of body fat; FFBFM, fat-free, bone-free mass; FM, fat mass; FST, 2-d fast; LMF, limit fed; P, prone; S, supine.

Rats were randomized to the first scan position, prone or supine. Subsequent scans alternated positions. The rat was weighed immediately after each scan and then repositioned for the next scan. Each DXA scan took 12 to 14 min to complete depending on the length of the rat. Breathing and other vital signs were checked visually to monitor the depth of anesthesia. Upon completion of the final scan, the rat was killed with a lethal dose of the mixture of ketamine and xylazine.

Chemical Analysis

After body weight was measured, the fur was removed with a small-animal electric shear. The weight of the rat without hair was recorded. Rats were frozen at −20°C. The completely frozen carcass was weighed again, sectioned into quarters, and ground in a commercial meat grinder into coarse, uniform particles. The coarsely ground carcass was weighed again and then freeze dried for a minimum of 3 d or until a constant weight was achieved.

The freeze-dried carcasses were transported to the proximate analysis laboratory for chemical analyses. The carcasses were ground again with a 0.95-mm plate to produce a finely powdered homogenate for subsequent chemical analyses. Triple, randomly selected aliquots (~2.0 g) were used for analyses. The aliquots were oven dried at 105°C to ensure thorough and complete dessication of the samples. All chemical analyses were done according to standards established by the Association of Official Analytical Chemists.[14] Total protein was determined by the micro-Kjeldahl nitrogen method, total fat by chloroform–petroleum ether extraction,[15] and ash by complete oxidation of each sample in a muffle furnace for 8 h at 550°C. The precision of the assays was 2%. The sum of the chemically derived components was 100 ± 1% (mean ± standard error of the mean) of intact body weight.

TBW was determined as the difference between the pre- and post–freeze-dried weights plus any moisture removed during the oven-drying process. FFBFM was the sum of TBW and protein. Percentage of body fat (%FAT) was calculated as fat mass divided by body mass (FM/BM) and expressed as a percentage.

Statistical Analysis

Data are presented as mean ± standard error of the mean. Analysis of variance with a repeated-measures design was used to determine the main effects of method (DXA versus chemical analysis, or CHEM) and position on body mass and soft-tissue composition.[16] When a significant main effect or an interaction was found, comparisons among methods, including position and treatments, were made with Tukey–Kramer post hoc tests, with adjustments for multiple comparisons.[16]

The method of Bland and Altman[17] was used to further assess the agreement between methods. Differences between individual, chemically determined, compositional values and individual DXA determinations were plotted against the mean of the two values to determine bias.

RESULTS

The reproducibility of repeated DXA determinations of BM and soft-tissue composition was similar in the prone and supine positions (Table I). Reproducibility was greatest, as shown by the lowest coefficient of variation (<1%), for BM. Intermediate levels of reproducibility (<1.5%) were observed for FFBFM determinations. The greatest variability was found in the DXA determinations of FM and %FAT, with coefficients of variation ranging from 2.6% to 8.9% and from 3.5% to 6.8%, respectively.

DXA determinations of soft-tissue composition were significantly correlated with reference chemical measurements (Table II). The coefficients of determination (R^2) relating body-composition variables assessed with prone and supine DXA scans and reference chemical values were similar. The variability (standard error of the estimate) in the linear relationships comparing chemical analyses and DXA determinations by position was similar.

A significant interaction was found between method and dietary treatment in the determination of BM (Table III). DXA significantly underestimated BM in all but the FAT group. Prone and supine DXA scans produced similar values of BM. The magnitude of the differences by method was relatively small (1% to 2%) compared with the differences by dietary treatment (27% to 30%).

Determination of FFBFM also was influenced by a significant interaction between method and treatment (Table IV). With the exception of the LMF rats, DXA significantly underestimated FFBFM. There was no significant effect of position on the DXA determinations of FFBFM. Differences by method and treatment were small (1%).

DXA significantly overestimated FM in the control, LMF, and DH groups (Table V). It produced similar FM values for the FAT and FST groups. DXA significantly overestimated %FAT in the LMF and DH groups and produced similar %FAT values in the FAT and FST groups (Table VI).

The bias, or absolute error, calculated as the DXA determination minus the CHEM reference value, tended to be small and related to the experimental treatment (Table VII). DXA significantly underestimated (e.g., DXA − CHEM < 0) BM for all treatment groups but only FFBM for the FST, LMF, and DH groups. In contrast, DXA significantly overestimated FM for all

TABLE II.

SUMMARY OF CORRELATION OF COEFFICIENTS RELATING DUAL X-RAY ABSORPIOMETRIC AND CHEMICAL ANALYSES OF SOFT-TISSUE COMPOSITION BY TREATMENT

			Variable							
			Body mass		FFBFM		FM		%FAT	
Treatment	*n*	Position	R^2	SEE	R^2	SEE	R^2	SEE	R^2	SEE
Control	15	Prone	0.982	1.36	0.906	8.23	0.352	3.32	0.933	0.76
	15	Supine	0.998	1.40	0.912	7.92	0.468	3.19	0.974	0.75
FAT	7	Prone	0.999	0.90	0.982	3.91	0.943	3.39	0.878	0.93
	7	Supine	0.999	1.01	0.969	2.27	0.896	4.56	0.771	1.25
FST	8	Prone	0.996	1.76	0.978	3.61	0.828	2.99	0.685	0.90
	8	Supine	0.996	1.95	0.978	3.53	0.814	3.03	0.688	0.90
LMF	10	Prone	0.998	0.85	0.837	6.74	0.371	3.12	0.658	1.02
	10	Supine	0.998	0.88	0.849	6.34	0.477	2.85	0.682	0.99
DH	9	Prone	0.999	1.07	0.952	6.64	0.595	3.36	0.903	0.79
	9	Supine	0.999	1.27	0.934	7.84	0.442	3.94	0.901	0.81

DH, treated with furosemide; FAT, high-fat diet; %FAT, percentage of body fat; FFBFM, fat-free, bone-free mass; FM, fat mass; FST, 2-d fast; LMF, limit fed; R^2, coefficient of determination; SEE, standard error of the estimate or mean square error.

treatment groups, except FST, and overestimated %FAT in all groups. These errors were significantly different from zero. The relative errors in DXA determinations also tended to be small and ranged from less than 1% for FFBFM, 1% to 2% for BM, approximately 2.5% for FFBFM, to 3% to 25% for FM and %FAT (Table VII).

Because body position during DXA scans did not significantly affect measurements of BM or soft-tissue composition, we averaged the prone and supine scan determinations to assess the relationships between errors and the various compositional variables. The errors, or bias, were independent of the range of the size of the various compositional determinations (Fig. 3). The slope of each relationship was similar ($P > 0.05$) to zero and the intercepts were not different from zero ($P > 0.05$), except for BM ($P < 0.05$).

DISCUSSION

The use of DXA for routine assessment of soft-tissue composition of laboratory rats is appealing because it is non-destructive and permits longitudinal assessments. The validity of DXA to identify

TABLE III.

EFFECTS OF POSITION AND TREATMENT ON BODY MASS (GRAMS) OF RATS

		Method*		
Treatment	*n*	CHEM	DXA-P	DXA-S
Control	15	358^{f}	355^{e}	356^{e}
FAT	7	376^{g}	374^{g}	375^{g}
FST	8	350^{d}	344^{c}	344^{c}
LMF	10	295^{b}	289^{a}	290^{a}
DH	9	359^{f}	355^{e}	355^{e}
Analysis of variance				
Root MSE				1.1
Method				$P < 0.001$
Treatment				$P < 0.001$
Method × treatment				$P < 0.001$

* Values with different superscripts are significantly different ($P < 0.05$).

CHEM, chemical analysis; DH, treated with furosemide; DXA-P, dual x-ray absorptiometry in the prone position; DXA-S, dual x-ray absorptiometry in the supine position; FAT, high-fat diet; FST, 2-d fast; LMF, limit fed; MSE, mean square error.

TABLE IV.

EFFECTS OF POSITION AND TREATMENT ON FAT-FREE, BONE-FREE MASS (GRAMS) OF RATS

		Method*		
Treatment	*n*	CHEM	DXA-P	DXA-S
Control	15	304^{defg}	300^{defg}	303^{fg}
FAT	7	300^{bcd}	297^{bcd}	299^{def}
FST	8	293^{bc}	291^{b}	293^{bc}
LMF	10	257^{a}	252^{a}	252^{a}
DH	9	301^{defg}	297^{bcd}	298^{cde}
Analysis of variance				
Root MSE				4.1
Method				$P < 0.024$
Treatment				$P < 0.002$
Method × treatment				$P < 0.041$

* Values with different superscripts are significantly different ($P < 0.05$).

CHEM, chemical analysis; DH, treated with furosemide; DXA-P, dual x-ray absorptiometry in the prone position; DXA-S, dual x-ray absorptiometry in the supine position; FAT, high-fat diet; FST, 2-d fast; LMF, limit fed; MSE, mean square error.

TABLE V.

EFFECTS OF POSITION AND TREATMENT ON FAT MASS (GRAMS) OF RATS

		Method*		
Treatment	*n*	CHEM	DXA-P	DXA-S
Control	15	42.7[a]	47.9[b]	45.6[ab]
FAT	7	65.9	70.2	69.6
FST	8	43.2	45.5	43.2
LMF	10	23.7[a]	29.9[b]	30.4[b]
DH	9	44.2[a]	50.9[b]	50.2[b]
Analysis of variance				
Root MSE				3.6
Method				$P < 0.001$
Treatment				$P < 0.001$
Method × treatment				$P < 0.223$

* Values with different superscripts are significantly different ($P < 0.05$).
CHEM, chemical analysis; DH, treated with furosemide; DXA-P, dual x-ray absorptiometry in the prone position; DXA-S, dual x-ray absorptiometry in the supine position; FAT, high-fat diet; FST, 2-d fast; LMF, limit fed; MSE, mean square error.

alterations in body composition relative to changes in energy balance and hydration is unknown. The reproducibility of DXA determinations of FFBFM (<1.3%), FM (<9%), and %FAT (<7%) in controlled conditions of nutritional perturbation was high despite repositioning errors and is similar to, if not greater than, previously published estimates of DXA precision of soft-tissue determination in rats.[10,11] Although DXA determinations of BM and soft-tissue composition were significantly correlated with chemical reference values, DXA significantly underestimated BM and FFBFM and overestimated FM and %FAT. These findings are consistent with previous reports in rats without nutrition or other interventions.[10,11]

TABLE VI.

EFFECTS OF POSITION AND TREATMENT ON BODY FATNESS (%) OF RATS

		Method*		
Treatment	*n*	CHEM	DXA-P	DXA-S
Control	15	11.9[a]	13.5[b]	12.8[bc]
FAT	7	17.4	18.6	18.4
FST	8	12.3	13.2	12.4
LMF	10	8.0[a]	10.3[b]	10.4[b]
DH	9	12.3[a]	14.3[b]	14.1[b]
Analysis of variance				
Root MSE				1.0
Method				$P < 0.001$
Treatment				$P < 0.001$
Method × treatment				$P < 0.120$

* Values with different superscripts are significantly different ($P < 0.05$).
CHEM, chemical analysis; DH, treated with furosemide; DXA-P, dual x-ray absorptiometry in the prone position; DXA-S, dual x-ray absorptiometry in the supine position; FAT, high-fat diet; FST, 2-d fast; LMF, limit fed; MSE, mean square error.

TABLE VII.

BIAS AND RELATIVE ERROR IN DXA DETERMINATION OF SOFT-TISSUE COMPOSITION OF 49 RATS

	Treatment				
Variable	Control	FAT	FST	LMF	DH
Mass (g)	−3.1†	−1.4†	−6.5†	−6.2†	−4.5†
	−0.7%‡	−0.4%	−1.7%	−1.4%	−1.1%
FFBFM (g)	−1.2	−0.9	−3.6†	−6.0†	−4.9†
	+0.8%	−0.7%	−0.7%	−2.3%	−1.8%
FM (g)	+5.2†	+4.2†	+2.8	+6.0†	+6.3†
	+7.1%	+6.1%	+2.8%	+25.0%	+14.5%
Fat (%)	+1.5†	+1.1†	+1.0†	+2.2†	+1.9†
	+12.1%	+6.3%	+8.1%	+25.0%	+15.4%

* Bias = DXA − CHEM.
† $P < 0.05$; H_0: bias = 0.
‡ 100% ([DXA − CHEM]/CHEM).
CHEM, chemical analysis; DH, treated with furosemide; DXA, dual x-ray absorptiometry; FAT, high-fat diet; FFBFM, fat-free, bone-free mass; FM, fat mass; FST, 2-d fast; LMF, limit fed.

Importantly, DXA clearly discriminated the effects of high-fat diets, hypocaloric diets, and acute and short-term dehydration on BM and soft-tissue composition. This finding is consistent with the observation that DXA was similar to CHEM in detecting gross differences in FM and protein contents between lean and obese rats.[11] As confirmed in the present study, Rose et al.[11] showed significant correlations between DXA and CHEM for soft-tissue composition and concluded that body composition can be estimated reliably with DXA in rats.

Any explanation for the discrepancies in determination of FM and %FAT between DXA and reference methods is not readily apparent. Jebb et al.[10] suggested that the use of stearic acid and water as the standards for the calibration of soft-tissue composition is inappropriate for x-ray attenuation of soft tissue in vivo. If this suggestion is accurate, changes in the chemical standards for soft-tissue–composition determination should overcome this limitation.

An alternate explanation is the orientation of the component in the path of the x-ray beam. Optimally, soft-tissue composition should be determined in areas without bone.[18] Because bone is a component of all body regions, algorithms for the assessment of soft-tissue composition assume that the composition of the body adjacent and in front of the bone is similar to that behind the bone.[19,20] This assumption is problematic in areas such as the thorax because of the ribs.[21] To evaluate the hypothesis that soft-tissue orientation relative to bone can lead to errors in DXA determination of fat and FFBFM, we measured rats in the supine and prone positions. Although there were no differences in soft-tissue composition by body position with DXA regardless of nutrition intervention, the values were significantly different, albeit small on a relative basis, than reference values (Tables 3–6). Thus, body orientation apparently is not a critical factor explaining the differences between DXA and chemical determinations of soft-tissue composition. However, practical considerations, such as ease of placement of the rat in the prone position and a general lack of motion artifact of the limbs associated with movement of the

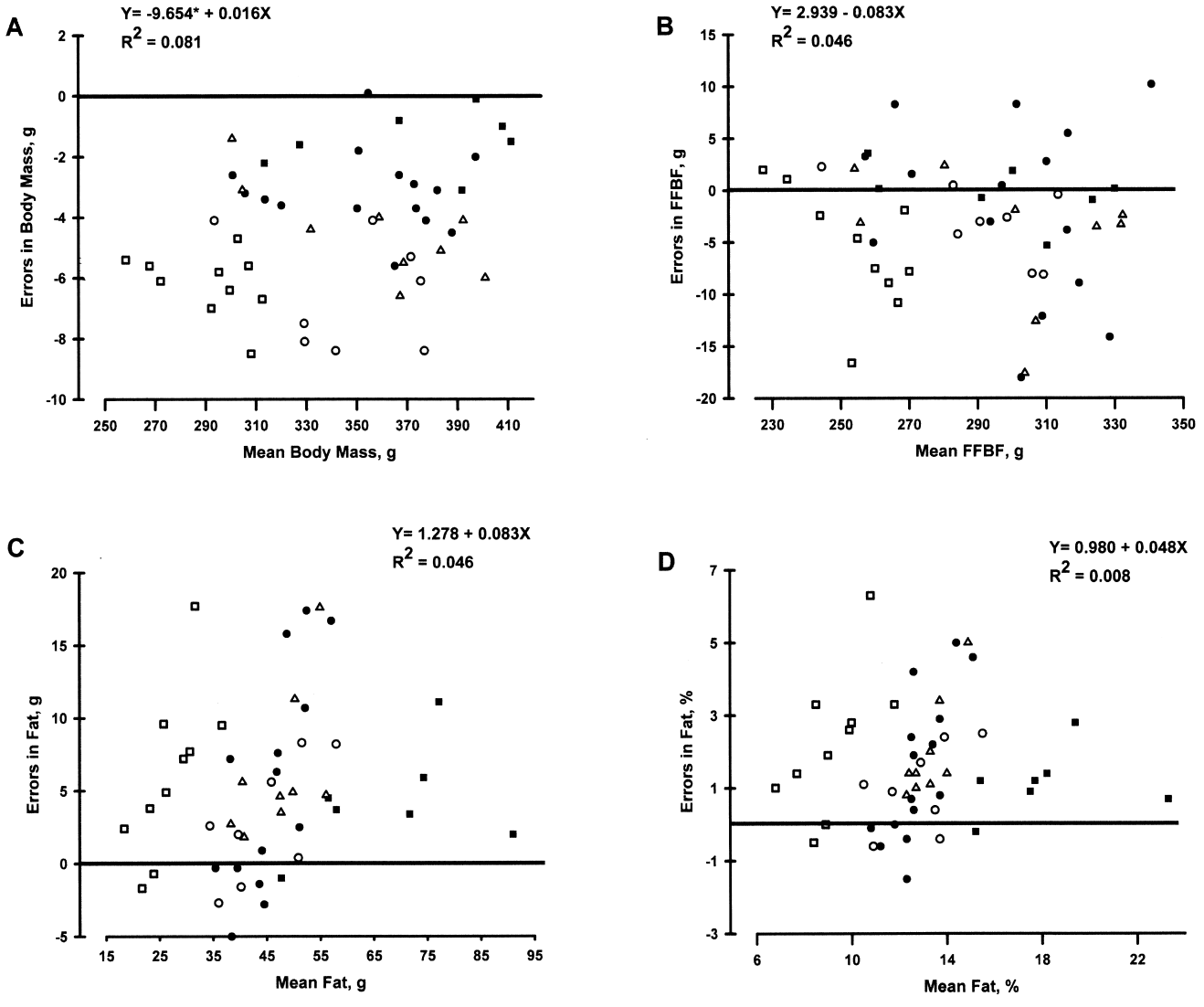

FIG. 3. Relationships between errors (dual x-ray absorptiometry and chemical analysis) and mean values of body mass and FFBFM (A and B) and fat mass and percentage of fat (C and D). *Intercept is significantly ($P < 0.05$) different from 0. FFBFM, fat-free, bone-free mass; solid circles, control; solid squares, fat fed; open circles, fasted 2 d; open squares, limit fed; triangles, treated with furosemide.

DXA table support the use of the prone placement of rodents during whole-body DXA scans.

We examined another potential source of error by evaluating the effect of body size on bias in DXA determination of body mass and composition. Although no significant relationships were found between errors (DXA − CHEM) and mean body-compositional variables (Fig. 3), no slopes of the regression lines differed from zero and only one intercept was significantly different from zero. Thus, DXA determinations for soft-tissue composition are independent of the magnitude of the measured variable (Hologic, Inc., personal communication).

We observed an interesting finding that may explain some of the discrepancy between the DXA and CHEM determinations of FAT and %FAT. Many of the rats urinated variable but appreciable amounts (2 to 8 g) that accumulated on their hair and pooled on the measurement platform during the repeated DXA scans. Thus, we speculate that extra corporeal fluids, that is the urine accumulated on the hair of the rat and the urine pooled on the measurement platform, explains some of the overestimation of FM and %FAT. Based on our measurements of urine volume (DXA bodyweight change during the DXA scan), the errors in DXA determinations of FM can be decreased approximately 1% to 2% when corrections are made for urine excretion.

Another complication may be the effect of dehydration on the estimates of %FAT. Diuresis was induced with the use of the anesthetic xylazine, which causes smooth-muscle relaxation.[22] Rose et al.[11] also used a mixture of ketamine and xylazine and reported a large discrepancy (20% to 30%) between DXA and CHEM determinations of FM and %FAT in rats. Interestingly, Jebb et al.[10] observed a similar error between DXA and CHEM in the measurement of %FAT with rats that had been killed and then frozen before the DXA measurement. These findings further support a possible adverse effect of extra corporeal fluid, excessive moisture on the hair of the rats, on the DXA determination of soft-tissue composition.

The error in the DXA determination of FM and %FAT in the DH group supports the postulated influence of dehydration on observed errors in DXA determinations of FM and %FAT. The DH and LMF groups had relatively increased dehydration (TBW/FFBFM; 67% and 68%, respectively) compared with the other groups (72% to 74%). In the DH group, the loss of fluid, a combination of the diuresis induced by the anesthesia and the

furosemide (9 to 24 g), presumably extracellular fluids, probably explains some of the errors. As discussed by Pietrobelli et al.,[23] decreases in the hydration of the fat-free body lead to overestimation of FM. These discrepancies, therefore, suggest that the current algorithms used to determine soft-tissue composition require revision when hydration levels diverge from the physiologic range (~73%).

The DXA method affords an advance over existing methods to assess soft-tissue composition of laboratory rats serially in response to dietary perturbations and interventions. Although theoretical assumptions involved in the derivation of algorithms based on x-ray attenuation for estimation of soft-tissue composition may be limiting at present, revisions in analysis software are needed to overcome errors associated with extra corporeal fluid and body-hydration status. Data from the present study establish the practical value of performing DXA scans in the prone position.

In conclusion, although DXA distinguished differences in soft-tissue composition of rats exposed to dietary and drug stressors, relatively small but significant differences were found in comparison with reference chemical analyses. Revisions of algorithms to calculate body fat are needed to accommodate variations in hydration status and improve DXA determination of soft-tissue composition.

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(For an additional perspective, see Editorial Opinions.)